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Quantitative Traits Breeding for Multifunctional Grasslands and Turf

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Chapter 4
Genetic Response to Climate Scenarios in *Dactylis* and *Festuca* of Temperate *Versus* Mediterranean Origin

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Abstract Climate change stresses the importance of exploring the genetic adaptation of plants to an increase of both temperature and water deficit. To this end, dense swards of Mediterranean and temperate cultivars of *Dactylis* and *Festuca* were seed harvested after 2 years under artificial climate scenarios (range of summer drought) in two environments (Mediterranean and temperate). To assess possible genetic changes, all populations of the next generation were assessed and compared with their parent population having not been subjected to these climate scenarios. The experiment was a spaced plant design in the temperate location. The results showed that a drought escape strategy through earlier heading time and reduced total plant yield was enhanced by scenarios of greater summer drought. However, the direction and the intensity of the response of the main measured traits suggested that the physiological plant adaptation to water stress differs between species. In *Dactylis*, the phenology appeared to be a very responsive trait in the late heading temperate cultivar and induced a plant size reduction, while no genetic variability seems to be still available for selection within the early flowering Mediterranean cultivar. By contrast, the balance between both traits was not found so crucial in *Festuca* which suggests a possible greater role of the rooting system in drought resistance for this species.

Keywords Population genetics • Perennial grasses • Adaptive response • Phenology • Drought tolerance

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Introduction

Climate change is raising new questions in genetics and breeding of forage crops. Models of plant production and climate projections in France (Terray et al. 2011), predict an increase of temperature beneficial to annual forage production in northern latitude because of growth enhancement in spring and autumn. Conversely, more severe summer drought will threaten more frequently any forage production in southern latitude in particular in the Mediterranean area (Durand et al. 2011). At this scale, Gordo and Sanz (2010) showed that many taxonomic groups are able to tune their phenology according to temperature variation over years as a plastic response. Investigating aerial biomass and senescence among ecotypes of various origins in four grass species submitted to extreme climate manipulations, Beierkuhnlein et al. (2011) underlined the importance of between and within genetic variability against interspecific variability in plant response to climate.

Most forage crops are out-breeding plants. As a consequence ecotypes as well as bred synthetic varieties necessarily harbour a minimum amount of genetic variability which is a prerequisite for selection to occur. However, individual plant reaction to water deficit involves many successive physiological processes over time which relationships and relative effects among and between species are still unclear in terms of genetics. In particular, it is unknown whether climate stressors may act as selection pressures in a dense sward of perennial grasses liable to heritable genetic changes over generations.

Dactylis sp. and *Festuca* sp. are good plant models to investigate these issues. Both complexes of species, as diploids in *Dactylis* and polyploids of high level in *Festuca*, are presently spread all over South-Europe having experienced strong climate variations in the course of evolution. Well-adapted ecotypes which have emerged, in particular in the Mediterranean area, are of great interest as genetic resources for breeding and to untangle the physiologic processes of plant adaptation to water deficit (Lelièvre et al. 2011). By applying experimentally climate scenarios in plots of *Dactylis* and *Festuca* of either temperate or Mediterranean origin, Poirier et al. (2012) showed that an annual water deficit of 500 mm clearly discriminates more between ecotype origins than between species with antagonistic relationships of annual productivity from the temperate cultivars vs. long term persistency from the Mediterranean ones.

In this context, it was interesting to couple the evaluation of agronomic response to climate scenarios with the quantification of possible genetic changes by using the same experimental plant material. This may be carried out longitudinally through plant sampling in a sward at successive periods of climate stress followed by new experimental assessment. However, this would not avoid all artefacts or bias due to plant cloning and limitation in sampling. Furthermore, this does not give access to the heritable part of the response liable to long term species evolution. We hereafter report the results of an evaluation in which all experimental plots were seed-harvested by the end of a 2-years long climate scenario experiment and all next generation populations were compared against the initial batch of seeds of each cultivar as control parent populations.

Material and Methods

In a preliminary experiment, two cultivars of temperate vs Mediterranean origin in *D. glomerata* and *F. arundinacea* were cultivated in Lusignan as a temperate site (lat. 46.4029716 – long. 0.0815821) and in Montpellier as a Mediterranean site (lat. 43.6379542 – long. 3.8623542) under 4 climate scenarios, either by increasing summer water deficit and/or by applying artificial heat waves in July (Poirier et al. 2012). In particular, the S1-scenario consisted in a control, holding water supplies at each site to its annual average (1980–2010). The S2-scenario halved water supplies by rain-shelter covering at night all year long. The cumulated water deficit (water supplies – evapotranspiration in spring and summer) which was achieved over the two first years in the scenario S1 and S2 amounted to −762 and −981 mm respectively in the temperate site and to −1,060 and −1,252 mm respectively in the Mediterranean site. Summer survival rate differentiated strongly between origin and site by decreasing down to 20 % in the temperate cultivars under the S2-scenario at the Mediterranean site. This did not prevent, however, to seed-multiply in-situ each experimental plot in spring of year-3 following almost complete recovery of the swards in the previous autumn. The 64 resulting populations of next generation (4 cultivars × 2 sites × 2 scenarios × 4 replicates (site × scenario)) as well as the 4 parent populations were established in a field nursery of spaced plants in the temperate site. The design was a split-plot in which the species was ascribed to the whole-plot, each of them further arranged in a randomized 3-complete blocks design of 32 next generation populations and 2 parent populations. The single plots were rows of 10 plants; in order to keep the same accuracy of mean estimates between next generation and parent populations, those latter were randomly planted into 3 rows × 10 plants each block. Following establishment in autumn, heading time was recorded in spring of the next year as the date of 2-spikes emergence and expressed in thermal time (°C × day since the 1st of January).

As a parallel second experiment, all next generation and parent populations were transplanted, following germination on March 1st, in 20-cm deep containers filled with compost. After 3-months of growing in glasshouse and no watering limiting conditions, all plants were clipped to 5 cm stubble height and aboveground biomass dried to estimate total plant dry matter yield. Before cutting, the lamina length of the last emerged adult leaf and the total plant height by stretching the leaves were measured. The design was a split-plot similar to the field nursery except that the replications in each site x scenario combination were randomly confounded with the present blocks in the design. Each species as a whole-plot was thus arranged into a randomized 4-complete blocks design of 8 next generation populations (2 cultivars × 2 sites × 2 scenarios) and their 2 parent populations. The blocks consisted of single containers of 10 randomized rows (populations) × 10 plants, each about 10 cm apart, surrounded by bordering plants.

Plant Material

The four cultivars which were used are registered on the French national list of varieties (http://www.herbe-book.org), both dactylis cultivars Ludac and Medly and the tall fescue cv. Centurion in 1997 while the other tall fescue cv. Soni in 2000. Medly is a typical very early flowering tetraploid *D. glomerata* cv. of Mediterranean origin bred from 9 entries from Australia × South France hybrids and Morocco which, however, the contribution between *hispanica vs glomerata* ssp. was not well defined. Centurion is a hexaploid *F. arundinacea* cv of 7 entries from Israel, Tunisia and Morocco, quite genetically distant from its temperate relative as crosses between the two origins give only sterile F1-hybrids (Jadas-Hécart and Gillet 1978).

Statistical Analyses

The data recorded in both trials were computed as 68 populations (4 cultivars × (16 next generation populations + the parent population)) under a mixed model of analysis of variance and holding all bloc interactions and plant within plot variation as random effects. The *proc mixed* procedure of SAS (1999) was used as well as the *lsmean*, *estimate* and *contrast* statements to test significance of the site × scenario genetic response within each cultivar. The responses were expressed as a standardized way (i.e. in standard deviation units, σ) by dividing the contrast of the mean of the next generation populations and that of the parent population by the residual term of Anova as an estimate of the phenotypic variance within cultivar. The responses were also fitted to regression models, nested or not within cultivar, by using the 2-years water deficit in each site × scenario as a regressor.

Results and Discussion

Ecotype differentiation strongly contrasted across species (Table 4.1). Expressed in standard deviation units, heading date differed by 4.28 σ between the Mediterranean and the temperate parent population in *Dactylis* while by 0.36 σ for above ground biomass. Both traits differentiated in a more balanced way within *Festuca* (−1.05 σ *vs* 0.99 σ resp.).

Furthermore, the traits correlated in an opposite way across species; earlier heading time was associated with less above ground biomass in *Dactylis* while in the early flowering Mediterranean *Festuca* all growth traits were higher than those in the temperate cultivar.

It is likely that variable chilling temperature in glasshouse during spring enabled the *Festuca* cv. of Mediterranean origin to exhibit higher plant above ground biomass than the temperate cv. A negative response of heading time in all populations of next generations was found, on average, over site × climate scenarios while

Table 4.1 Mean value of the traits recorded in the parental population of four cultivars of either temperate or Mediterranean origin in *D. glomerata* and *F. arundinacea* (interval of confidence at $P<0.05$ in brackets)

	Dactylis		*Festuca*	
Traits	Temperate	Mediterranean	Temperate	Mediterranean
Heading date (°C×d)	700	444	755	692
	[682–718]	[426–462]	[737–772]	[674–709]
Above ground biomass (mg DM plant^{-1})	1,319	1,168	819	1,235
	[1,095–1,543]	[945–1,390]	[591–1,048]	[1,012–1,458]
Plant height (mm)	668	563	534	600
	[633–703]	[529–598]	[499–569]	[565–634]
Adult lamina length (mm)	430	395	381	434
	[409–471]	[364–425]	[350–412]	[403–464]

above ground biomass responded positively in the plants from the temperate site and negatively in those from the Mediterranean site (Fig. 4.1). Negative regression clearly suggests that a drought escape strategy is primarily enhanced by increasing water shortage. Because of higher accuracy estimates, the response of heading time was found significantly different across cultivars (F 7:48 $df=2.907$; $P<0.0120$). Only a unique linear response ($r^2=0.747$; F 1:14 $df=41.23$; $P<0.0001$) was found in above ground biomass while heading time fitted better to a quadratic model ($r^2=0.972$; F 2:13 $df=227.31$; $P<0.0001$) emphasizing a particularly strong decrease from the temperate *Dactylis* grown under the S2-scenario in the Mediterranean site. By contrast, the Mediterranean cultivar of *Dactylis* gave no significant response of heading time across all site×scenario combinations.

By plotting the response of both traits in the range of the origin differentiation within species, overall changes from the temperate cultivar of *Dactylis* seem to mimic to some extent the natural evolution which occurred in that species towards earlier heading date and lower growing rate (Fig. 4.2). It appears therefore significant that the Mediterranean cultivar at the extreme of the distribution of heading date did not display any further significant response for that trait as if no genetic variability was still available.

The response in tall fescue appeared more balanced in the same way as the differentiation between parent populations was less pronounced than in *Dactylis*. The Mediterranean cultivar of tall fescue reacted similarly to the temperate cultivar of *Dactylis* by adapting both its phenology and plant size. By contrast, the response within the temperate *Festuca* seems to involve more positively the traits associated with growth rate. Rooting is considered as a major trait controlling persistency in tall fescue. Possibly, positive response of above ground biomass in the temperate *Festuca* results from an indirect response to selection for early deep rooting conferring higher fitness when plants have to cope with water deficits later in summer.

Using polyethylene or cotton wrap to prevent pollen contamination between plots may have induced artefacts by increasing temperature in the days before seed harvest. It is also obvious that harvesting seeds from 3-years old swards do not

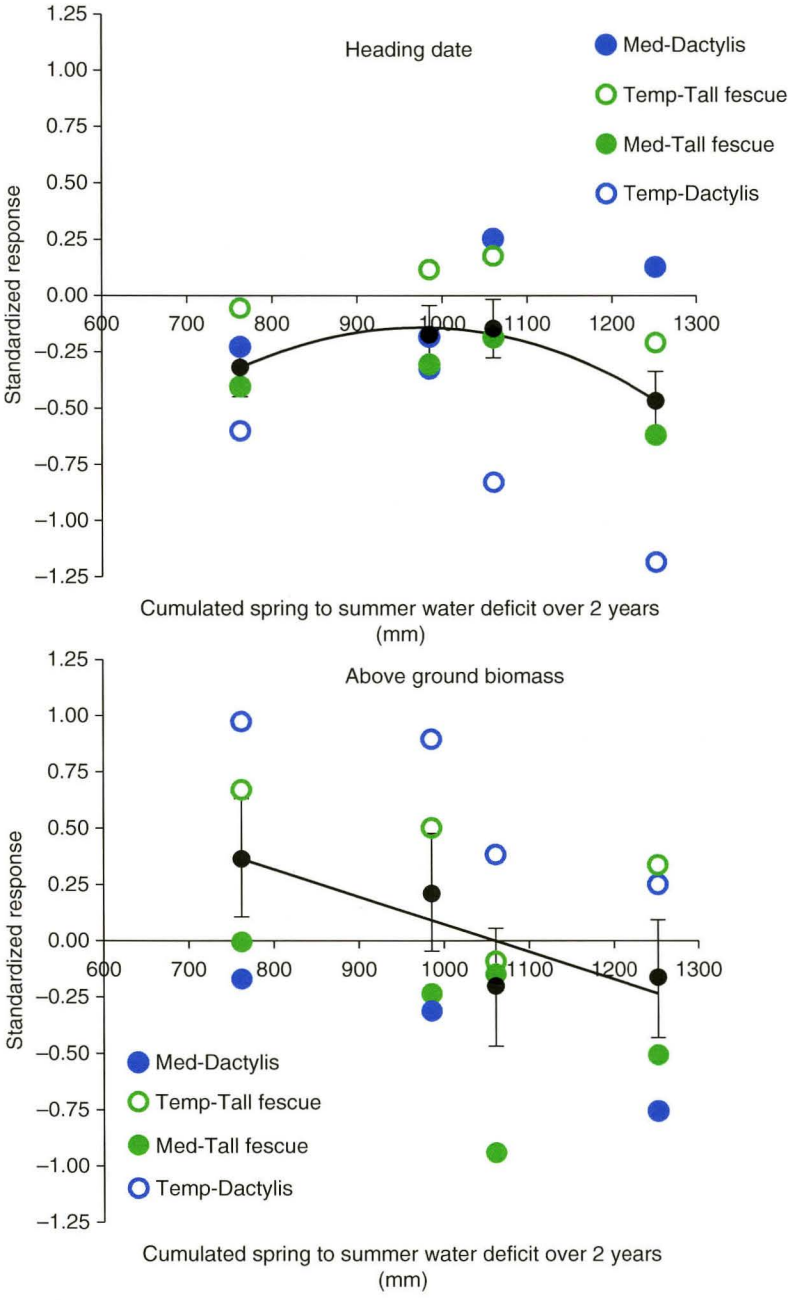

Fig. 4.1 Mean response of heading date and plant above ground biomass to 4 site×climate scenario combinations expressed against 2-years of cumulated spring to summer water deficit in 4 cultivars of either temperate or Mediterranean origin in *D. glomerata* and *F. arundinacea*

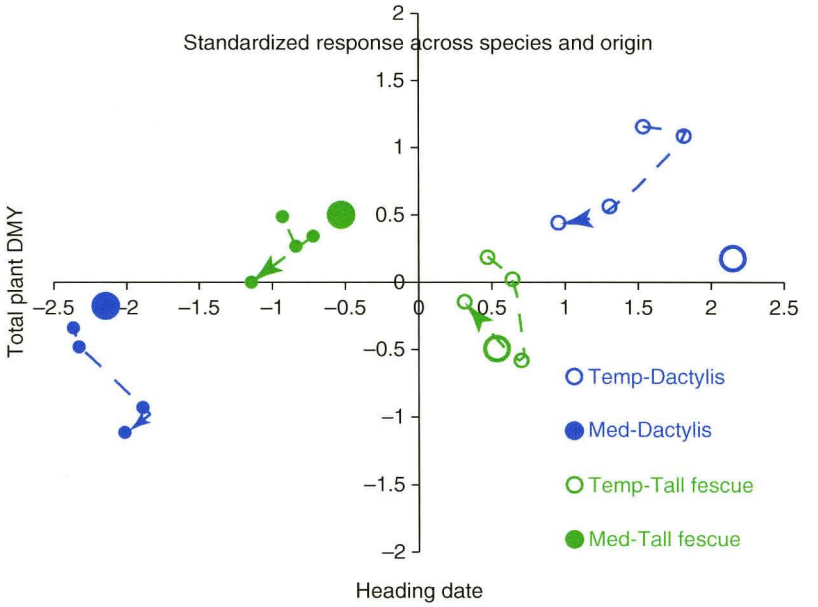

Fig. 4.2 Associated response of heading date and above ground biomass across site × climate scenarios in Mediterranean and temperate cultivars of *D. glomerata* and *F. arundinacea*. The symbol of the parent population of each cultivar is of larger size; the arrows rank the response of the next generation populations across scenario × site according to increasing water deficit, from S1-scenario × temperate site to S2-scenario × Mediterranean site

include only response to water deficit. In this respect, positive response of above ground biomass under the least water limiting scenario in the temperate site could result from a response for competing ability, expressed at early stage of plot establishment and/or during recovery in autumn. In any case, the results show that substantial genetic response can result from genetic variability restricted to a unique cultivar as a parent population. Mean standardized response about 0.5 σ in the absolute implies that true mortality occurred in swards despite apparent overall recovery and that it was accompanied by strong individual selection (e.g. 10 % of rate of selection assuming moderate heritability *s.s.* of 0.30). In some instances, the responses which were observed are orthogonal to species and/or ecotype within species differentiation which suggests that all traits are not of the same importance in plant responses to water stress and they are under complex non-linear control. In particular, it clearly appears that phenology in *Festuca* would not be so crucial for climate adaptation as it is in *Dactylis* or, at least, that it may act secondarily once sufficient deep rooting is achieved through natural selection as seen from the Mediterranean *Festuca* cultivar.

As the response to water deficit prioritizes an escape strategy, to aim at more productivity or, at least, maintaining a same level while maintaining persistency remains a challenge for plant breeders. In this respect, refinement in the climate change models will help to better define forage ideotypes of productivity *vs* persistency alternative in terms of annual climate risk. Future work is presently undertaken to estimate the relative role of phenology in *Dactylis* and *Festuca* drought tolerance from crosses between and within origins. Molecular DArT markers are simultaneously developed to assist the assessment of genetic response as well as to breed for ideotypes (Ghesquière et al. 2012). In this respect, *Dactylis* provides a good genetic model because of total cross-compatibility within the whole complex of species. Moreover, strong origin differentiation and response which were recorded suggest that strong linkage disequilibrium with molecular markers could exist thus facilitating the detection of further genetic changes as well as breeding for a better adaptation from inter-origin crosses.

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